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Vitamin D_3, gamma interferon, and control of proliferation of *Mycobacterium tuberculosis* by human monocytes

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SUMMARY

Previous studies have shown that recombinant interferon gamma (IFN-γ), crude T cell supernatants, or appropriate T-cell lines can cause total inhibition of the growth of *M. tuberculosis* inside murine peritoneal macrophages. In similar experiments with human monocytes much smaller effects are seen. This could be due to the relative immaturity of these cells. Because dihydroxy vitamin D_3 (1,25-$(OH)_2$ D_3) can cause phenotypic differentiation of immature leukaemic lines into macrophage-like cells, we have explored the possibility that exposure to cholecalciferol metabolites *in vitro* might increase the ability of monocytes to control proliferation of *M. tuberculosis*, or cause monocytes to mature into cells able to respond appropriately to IFN-γ. Incubation of monocytes with three cholecalciferol metabolites induced anti-tuberculosis activity to an extent that correlated with their binding affinities to the intracellular receptor protein for the derivatives. 1,25-$(OH)_2$ D_3 also primed monocytes for phorbol myristate acetate-triggered reduction of nitroblue tetrazolium. The effects were additive rather than synergistic with those of IFN-γ. Monocytes incubated with IFN-γ developed 25-OH D_3 1-hydroxylase activity, detected by conversion of tritiated 25-(OH) D_3 to a more polar metabolite which coeluted with 1,25-$(OH)_2$ D_3 on straight and reverse-phase HPLC. The latter is a more active form *in vivo*. These findings help to explain claims for the efficacy of vitamin D in the treatment of some forms of tuberculosis, and also the occasional finding of raised serum calcium, and disturbed vitamin D metabolism in these patients.

INTRODUCTION

When murine peritoneal macrophages infected with *Mycobacterium tuberculosis* are activated by lymphokine-rich supernatants from T cells stimulated with concanavalin A, or by direct addition to the macrophage cultures of tuberculin-responsive T-cell lines, they develop the ability to cause marked inhibition of the proliferation of the mycobacteria (Rook *et al.*, 1985a). The degree of inhibition reaches a plateau at a level which is compatible with the hypothesis that 100% stasis is being achieved. Subsequent studies of the interaction between lymphokines and a bactericidal antibiotic have confirmed that the effect is indeed stasis, rather than kill of some organisms balanced by proliferation of others (Altes *et al.*, 1985). These results imply that essentially all the macrophages in a murine peritoneal population can be activated to control *M. tuberculosis*. We therefore attempted to repeated these observations using human macrophages from sources, activated by human lymphokine, or recombinant interferon gamma (IFN-γ) (Rook, 1985). The efficacy of fresh peripheral blood monocytes was variable and inconsistent, but macrophages derived from monocytes by culture *in vitro* for 4–5 days showed a reproducible capacity for lymphokine-enhanced inhibition of *M. tuberculosis* (Rook, 1985). However, even in these cells, the effect reached a plateau at a level that indicates much less capacity for activation of anti-mycobacterial mechanisms than had been seen in the murine peritoneal macrophages. One possible explanation for this is the existence in man of functionally distinct subpopulations of macrophages at different stages of maturation. Heterogeneity of human monocytes in their ability to develop anti-microbial activity following exposure to IFN-γ has been observed with *Leishmania donovani* (M. S. Meltzer, personal communication).

1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2$ D_3), the hormonally active form of vitamin D_3, can cause maturation of murine myeloid leukaemia cells (Abe *et al.*, 1981), and of the human monocytic line U937 (Amento *et al.*,1984), and phenotypic differentiation of the promyelocytic leukaemia cell line HL60 into granulocytes (Miyaura *et al.*, 1981) or macrophage-like cells (Mangelsdorf *et al.*, 1984). It therefore seemed possible that exposure to 1,25-$(OH)_2$ D_3 might either directly modify the ability of monocytes to control *M. tuberculosis*, or regulate the proportion of the monocytes able to respond appropriately to lymphokines. We therefore examined the ability of a number of metabolites of vitamin D_3, including 1,25-$(OH)_2$ D_3, to modify

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handling of *M. tuberculosis* by human monocytes, with and without IFN-γ. Interactions between 1,25-$(OH)_2$ D_3 and IFN-γ were also studied by measurement of phorbol myristate triggered reduction of nitroblue tetrazolium and the effect of IFN-γ on the 25-hydroxy vitamin D-1-hydroxylase enzyme.

MATERIALS AND METHODS

Culture of human monocytes

Blood from normal volunteers was taken into 60-ml syringes (Becton-Dickinson, Cowley, Oxon) and defibrinated by shaking vigorously for 10 min with six to10 glass beads (6 mm diameter) in two 30-ml glass universal bottles. Use of defibrinated rather than heparinized blood avoids adherence of platelets to the macrophages. Defibrinated blood was then centrifuged for 5 min at 1000 ***g*** to allow collection of the serum. The cells were then resuspended to twice the original volume with RPMI-1640 (Gibco, Paisley, Renfrewshire), layered over Ficoll–Hypaque (Flow Laboratories, Irvine, Ayrshire) and centrifuged at 400 ***g*** for 30 min. The cells from the interface were washed once in RPMI-1640, and then suspended in 20 ml of the same medium supplemented with 10% autologous serum, and allowed to settle in tissue culture flasks (80 sq cm, Nunc, Roskilde, Denmark) for 2 hr at 37°. Then, the non-adherent cells were washed out with three changes of RPMI, and the medium replaced with 20 ml of 10% autologous serum in RPMI containing 3·3 mg/ml disodium EDTA. The flasks were incubated for 40 min at 37° and the detached cells poured off into a plastic universal. If necessary, EDTA-containing medium was squirted at the bottom of the flask with a pasteur pipette to detach the remaining cells. The harvested cells were then centrifuged once at 150 ***g*** and resuspended in 20% autologous serum in RPMI-1640 without antibiotics. They were plated in flat-bottomed microtitre wells (Nunc, 1-6700 B) at 10^5 per well in 0·2 ml of medium.

1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2$ D_3), 1,24,25-trihydroxy vitamin D_3 (1,24,25-$(OH)_3$ D_3), and 25-hydroxy vitamin D_3 (25-OHD_3), which were all the kind gift of Dr M. Uskokovic (Hoffman–La Roche, Nutley, NJ), were added at doses ranging from 1 nM to 10 μM to the cultures in a final concentration of 1% ethanol in RPMI. The same concentration of ethanol was added to control wells, although ethanol alone had no detectable effect in the experiments reported here. Recombinant IFN-γ (a gift from Boehringer, Vienna, Austria) was added at doses ranging from 1 to 300 U/ml in RPMI.

In order to check that vitamin D derivatives, and gamma interferon as used in these studies, did not have significant effects on the survival macrophages, replicate wells were washed with medium, and the live adherent cells lysed with 1% Tween 80 in 0·1 M citric acid. Nuclei were then counted in a haemocytometer. Circumstances under which gamma interferon affects cell survival are discussed elsewhere (Rook *et al.*, 1985b).

Assay for anti-mycobacterial effects

The assay was essentially as described elsewhere (Rook *et al.*, 1985a). Briefly, a suspension of *M. tuberculosis* was prepared from a fresh (1-week) culture of a clinical isolate on Lowenstein–Jensen medium. A loop of growth was suspended in 5 ml of RPMI by repeatedly forcing it out of a a 1-ml syringe jammed in the bottom of a conical 30-ml plastic universal. The suspension was centrifuged at 700 ***g*** for 2 min. Particles in the top 2 ml, representing one or a few bacteria, were counted underphase contrast in a platelet-counting chamber. One hundred thousand particles were added to each well in 25 μl of RPMI. After 4 days, one drop of 2% saponin (Sigma, Poole, Dorset) was added to each well to lyse the human cells, and supplements were added in a further 25 μl to facilitate the growth of surviving bacilli. Final concentratons were ferric ammonium citrate, 50 μg/ml, fungizone, 2·5 μg/ml, 0·2% sodium glutamate, and 0·2% L-asparagine. Finally, 0·25 μCi of [6–3H] uracil (Amersham International, Amersham, Bucks) was added in 20 μl of RPMI. After 48 hr, the cultures were visually assessed using inverted phase contrast and then harvested by suction through glass fibre filters (Whatman, Maidstone, Kent, type GFC) in a standard cell harvester, and counted by liquid scintillation spectrophotometry.

Assay of phorbol myristate-triggered reduction of nitroblue tetrazolium

Details of this improved quantitative assay for NBT reduction, and comparison with previously described techniques, are described elsewhere (Rook *et al.*, 1985b).

Assay of 25-hydroxy vitamin D_3-1-hydroxylase activity

Mononuclear cells were cultured in 3-cm wells (Nunc) at a density of 2×10^6 cells/well for 3 days in the presence of increasing doses of IFN-γ (10–300 U/ml) diluted in 20% autologous serum in RPMI. After 3 days, the cultures were depleted of serum by sequential washes in RPMI alone and then cultured for a further 3 hr with 5 nM 25-[26, 27-3H]-OH D_3 (specific activity 180 Ci/mmol, Amersham International) in 1·0 ml of RPMI containing 1% ethanol. Controls received ethanol only. The cells were then harvested with a rubber spatula, combined with the medium and disrupted by sonication. Immediately, 1·0 ml of acetonitrile was added and the lipid soluble sterols extracted on C 18 Sep-paks (Waters Associates, Millipore, Harrow, Middlesex). Material which coeluted with authentic 1,25-$(OH)_2$ D_3 was then isolated by sequential straight and reverse-phase hig-pressure liquid chromatography as described elsewhere (Fraher *et al.*, 1985).

RESULTS

Proliferation of *M. tuberculosis*

Monocytes were cultured with or without the metabolites of vitamin D_3 for three days, and then infected with *M. tuberculosis*. Four days later, the monocytes were lysed and [^{3}H]uracil incorporation by the bacilli assessed. Illustrated in Fig. 1 are the dose-response curves thus generated for three of the metabolites. 1,25-$(OH)_2$ D_3 caused inhibition of [^{3}H]uracil uptake over the range 10^{-5}–10^{-7} molar, with variable inhibition at 10^{-9} molar. The inhibition observed always plateaued from 10^{-7} molar upwards. When 25-OH D_3 was added to the cultures, significant inhibition occurred only at the highest concentration tested (10^{-5} M). However, when the trihydroxylated metabolite 1,24-25-$(OH)_3$ D_3 was added, the effects seen were intermediate with significant inhibition of uracil uptake occurring at a concentration of 10^{-7} M. In control experiments, none of the metabolites had any effect on the proliferation of *M. tuberculosis* in the absence of monocytes, nor any significant effect on cell numbers (data not shown).

In order study possible interactions between 1,25-$(OH)_2$ D_3 and IFN-γ on anti-mycobacterial activity, monocytes were

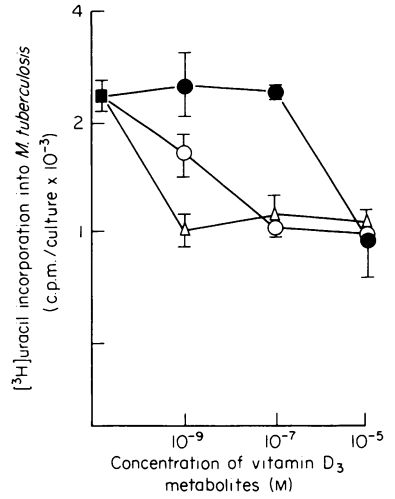

Figure 1. The effect of three cholecalciferol metabolites on the proliferation of *M. tuberculosis* in cultures of human monocytes. Confluent monocyte monolayers were cultured with the indicated concentrations of 25-(OH) D_3 (●), 1,24,25-$(OH)_3$ D_3 (○), or 1,25-$(OH)_2$ D_3 (△) for 3 days, and then infected with *M. tuberculosis* (10^5/well). Four days later, monocytes were lysed with saponin, and organisms were labelled with tritiated uracil. Results expressed as c.p.m./culture ± SD.

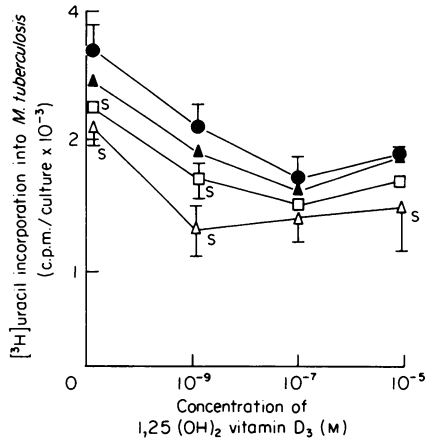

Figure 2. The effects of 1,25-$(OH)_2$ D_3 on the proliferation of *M. tuberculosis* in human monocytes without (●), or with 10 U/ml (▲), 100 U/ml (□), or 300 U/ml (△) of gamma interferon. Protocol as in the legend of Fig. 1. An 's' indicates significant inhibition by IFN-γ compared to the value for the macrophages cultured in the same concentration of the cholecalciferol metabolite, without interferon (Student's *t*-test).

incubated alone or with IFN-γ, 1,25-$(OH)_2$ D_3 or both at increasing doses for 3 days. The cells were then infected with *M. tuberculosis* and reincubated for a further 4 days before lysis and assessment of [^{3}H]uracil uptake. As seen in Fig. 2, increasing concentrations of IFN-γ alone inhibited the incorpation of uracil into the bacilli by up to 50% at a dose of 300 U/ml. When 1,25-$(OH)_2$ D_3 was also included in the cultures, further dose-related decreases of incorporation of the tracer were observed which again plateaued at 10^{-7} molar 1,25-$(OH)_2$ D_3. These results suggested that effects of 1,25-$(OH)_2$ D_3 and IFN-γ were additive rather than synergistic.

Nitroblue tetrazolium reduction

As reported previously (Rook *et al.*, 1985b) exposure of monocytes to IFN-γ for 3 days markedly increased the PMA-inducible reduction of NBT. Moreover, direct examination of the plate before the solubilization procedure revealed that 100% of the cells contained formazan. When 1,25-$(OH)_2$ D_3 was included in the cultures instead of IFN-γ, this also caused a dose-related increase in PMA-inducible reduction of NBT (Fig. 3). At the highest concentration used (10^{-5} M), 70% of the cells contained formazan. In order to test for interaction between the two agents, 1,25-$(OH)_2$ D_3 was added on Day 1, and IFN-γ on Day 4. PMA-induced reduction of NBT was assayed 3 days later. Under these conditions, the results (Fig. 3) suggested that the effects of the two agents were additive at submaximal concentrations of IFN-γ.

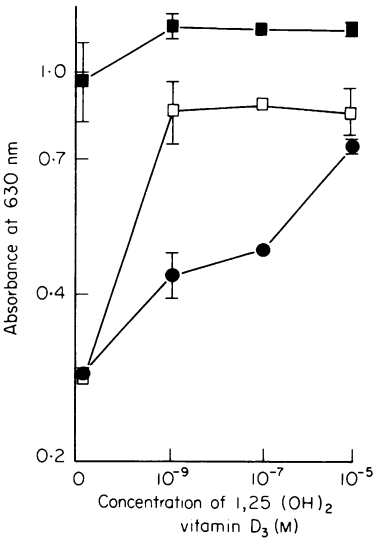

Figure 3. Priming of PMA-inducible reduction of nitroblue tetrazolium by exposure of monocytes to 1,25-$(OH)_2$ D_3 for 7 days, without gamma interferon (●), or with 10 U/ml (□), or 100 U/ml (■) added for the last 3 days.

Effect of IFN-γ on 25-OH D_3-1-hydroxylase activity

Following exposure for 3 days to increasing doses of IFN-γ, monocyte-derived macrophage cultures were depleted of serum and then incubated with tritiated 25-OH D_3 for 3 hr. When the products of these incubations were extracted and material which coeluted with authentic 1,25-$(OH)_2$ D_3 on both straight and reverse-phase HPLC isolated, striking effects of IFN-γ on the metabolism of 25-OH D_3 were observed. As shown in Fig. 4, in the absence of IFN-γ these cells were capable of metabolizing 3H-25-OH D_3 to a more polar metabolite which cochromatographed with authentic 1,25-$(OH)_2$ D_3, but the amounts produced could be increased significantly following preincubation of the cells with the lymphokine. At the maximum concentration of IFN-γ used (300 U/ml) the amounts of putative 1,25-$(OH)_2$ D_3 formed were approximately 25 fmol/10^6 cells/hr from a concentration of substrate of 5 nM.

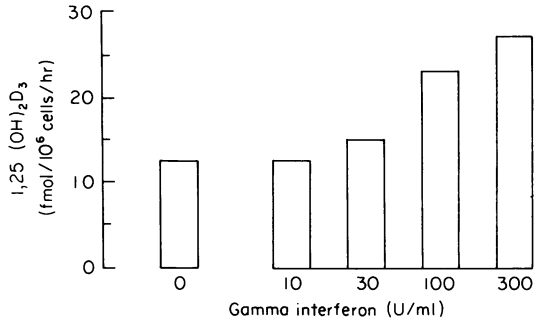

Figure 4. Augmentation of 25(OH) D_3-1-hydroxylase activity in human monocytes following exposure to the indicated concentrations of gamma interferon for 3 days. After exposure, the monolayers were washed, and incubated with tritiated 25(OH) D_3 for 3 hr. Conversion to 1,25-$(OH)_2$ D_3 was assayed as described in the text.

DISCUSSION

The results presented here indicate that hydroxylated metabolites of vitamin D_3 can cause inhibition of the growth of *M. tuberculosis* in normal human monocytes. Of the metabolites tested, 1,25-$(OH)_2$ D_3 was the most potent and was effective when added at concentrations similar to those which are capable of causing phenotypic differentiation of the HL 60 and U 937 promyelocytic leukaemia cell lines (Miyaura *et al.*, 1981; Amento *et al.*, 1984; Mangelsdorf *et al.*, 1984). The other metabolites tested were capable of causing inhibition of growth of the bacilli but at higher doses than for 1,25-$(OH)_2$ D_3 and, in order of effectiveness, displayed similarities to their binding affinities to the intracellular receptor protein for 1,25-$(OH)_2$ D_3. When 1,25-$(OH)_2$ D_3 and IFN-γ were included together in cultures, the effects seen were additive and of similar magnitude, with a combined incubation of 10^{-7} M 1,25-$(OH)_2$ D_3 and 300 U/ml IFN-γ causing greater than 60% inhibition of growth.

The present experiments do not reveal the mechanism. In the experimental system used, *M. tuberculosis* increases approximately eight-fold (three generations) in 4 days in human monocytes. This replication is intracellular because small inocula of *M. tuberculosis* grow very poorly in media rich in human serum unless an iron supplement is added (Kochan, 1973), and any organisms released by dying cells are rapidly rephygocytosed by other cells in high-density macrophage cultures (Rook *et al.*, 1985a; Altes *et al.*, 1985). Therefore, the decrease in [^{3}H]uracil uptake seen could be due to kill of a subpopulation of the bacilli, or reduction of the rate of replication from three generations to only two in 4 days.

When the two agents were tested for their ability to prime macrophages for PMA-induced reduction of NBT, additive effects were observed at submaximal concentrations of IFN-γ (below 100 U/ml). However, it is unlikely that the antimycobacterial effect of the 1,25-$(OH)_2$ D_3 was due to a stimulation of oxygen reduction, since it caused an additive antimycobacterial effect in the presence of concentrations of IFN-γ which independently caused a maximal stimulation of NBT reduction.

Since both 1,25-$(OH)_2$ D_3 and IFN-γ appear to enhance antimycobacterial effects and oxygen reduction, it is interesting that they have opposing effects on the phenotype of the cells, as determined by a range of monoclonal antibodies (L.W, Poulter, G.A.W. Rook *et al.*, in preparation).

Further interactions between IFN-γ and 1,25-$(OH)_2$ D_3 were observed by demonstrating that preincubation of the cells with increasing doses of IFN-γ led to increased ability to metabolize 3H-25-OH D_3 to a metabolite which coeluted with 1,25-$(OH)_2$ D_3 on multiple HPLC systems. This phenomenon has also been observed in macrophages cultured from broncho-alveolar lavage samples obtained both from normal subjects (Koeffler *et al.*, 1985) and from patients with sarcoidosis (Adams & Gacad, 1985, Fraher *et al.*, 1985). The 25-(OH) derivative is relatively inactive, but is the most abundant circulating form of vitamin D. The 1,25-$(OH)_2$ D_3 derivative is the most active. Therefore, if monocytes activated by IFN-γ are capable of expressing the 1-hydroxylase activity, it is possible that the effects of other vitamin D_3 metabolites in this system may be due to the production of 1-hydroxylated derivatives which then interact with the specific intracellular receptor.

These observations may help to explain some of the speculations and observations about the role of vitamin D in tuberculosis. Cod liver oil was first advocated for the treatment of tuberculosis in 1770, and it was widely used for this purpose in the nineteenth century. Many workers tried claciferol in the late 1940s, and rationalized this treatment by suggesting a role in the calcification of lesions. Numerous authors claimed that it was effective in the treatment of skin tuberculosis (Dowling & Prosser Thomas, 1946; Charpy, 1950), but in pulmonary disease the effect was equivocal, or even detrimental (Fanielle, 1951). The advent of anti-tuberculous agents in the mid 1950s led to a decline in this type of therapy. Nevertheless, it was claimed that both in guinea-pigs (Chmelev, 1959) and man (Brincourt, 1967) severe late disease which would be fatal if treated with tuberculostatic drugs alone, could be cured if calciferol was administered as well.

Subsequently, it was observed that some patients with tuberculosis have raised serum calcium levels, and that there can be an abnormal hypercalcaemic response to vitamin D administered with anti-tubercular therapy (Abassi *et al.*, 1979; Narang, Gupta & Jain, 1984). Recent work has confirmed the existence of an abnormality in the regulation of serum levels of 1,25 dihydroxy vitamin D_3 in patients (Epstein *et al.*, 1984).

The findings reported in this paper could explain these observations, because lymphokine-activated macrophages in the tuberculous lesions may convert 25(OH) D_3 to 1,25-$(OH)_2$ D_3, resulting in a beneficial increased anti-mycobacterial activity of a subpopulation of macrophages, but also causing changes in regulation of vitamin D and of calcium.

It remains to be proven that unusually high (Grange *et al.*, 1985) or low levels of circulating cholecalciferol, such as may occur when Asian immigrants arrive in Britain, affect susceptibility to the disease.

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REFERENCES

Abassi A.A., Chemplavil J.K., Farah S., Muller B.F. & Arnstein A.R. (1979) Hypercalcaemia in active pulmonary tuberculosis. *Ann. Intern. Med.* **90,** 324.

ABE E., MIYAURA C., SAKAGAMI H., TAKEDA M., KONNO K., YAMAZAKI T., YOSHIKI S. & SUDA T. (1981) Differentiation of mouse myeloid leukaemia cells induced by 1,25 dihydroxy vitamin D3. *Proc. natl. Acad. Sci.* U.S.A. **78,** 4990.

ADAMS J.S. & GACAD M.A. (1985) Characterisation of a 1-hydroxylation of vitamin D3 sterols by cultured alveolar macrophages from patients with sarcoidosis. *J. exp. Med.* **161,** 755.

ALTES C., STEELE J., STANFORD J.L. & ROOK G.A.W. (1985) The effect of lymphokines on the ability of macrophages to protect mycobacteria from a bactericidal antibiotic *Tubercle* (in press).

AMENTO E.P., BHALLA A.K., KURNICK J.T., KRADIN R.L., CLEMENS T.L., HOLICK S.A., HOLICK M.F. & KRANE S.M. (1984) 1,25 dihydroxyvitamin D3 induces maturation of the human monocyte cell line U937, and in association with a factor from human T lymphocytes, augments production of the monokine, mononuclear cell factor. *J. clin. Invest.* **73,** 731.

BRINCOURT J. (1967) Le caciferol a-t-il une action liquefiante sur le caseum. *Poumon-Coeur*, **23,** 841.

CHARPY J. (1950) Quelques traitements vitamines ou par substances fonctionnelles en dermatologie. *Bull. Med.* **24,** 505.

CHMELEV N.A. (1959) Les recidives de la tuberculose et le probleme du renforcement des preparations tuberculostatiques. *Bull. Un. int. Tuberc.* **29,** 683

DOWLING G.S. & PROSSER THOMAS E.W. (1946) Treatment of *Lupus vulgaris* with calciferol. *Lancet*, **i,** 919.

EPSTEIN S., STERN P.H., BELL N.H., DOWDESWELL I. & TURNER R.T. (1984) Evidence for abnormal regulation of circulating 1,25-dihydroxy vitamin D in patients with pulmonary tuberculosis and normal calcium metabolism. *Calcif. Tissue. Int.* **36,** 541.

FANIELLE G. (1951) La D-vitaminotherapie massive. *Bruxelles Med.* **31,** 1475.

FRAHER L.J., SANDLER L.M., KARMALI, R., BARKER S., FLINT K.C., HUDSPITH B.N., JOHNSON N.M. & O'RIORDAN J.L.H. (1985) The control of production of 1,25 dihydroxy vitamin D3 in man: studies of vitamin D deficiency and sarcoidosis. *Proceedings of the Sixth Vitamin D Workshop.* Walter de Gruyter, Berlin.

GRANGE J.M., DAVIES P.D.O., BROWN R.C., WOODHEAD J.S. & KARDJITO T. (1985) A study of vitamin D levels in Indonesian patients with untreated pulmonary tuberculosis. *Tubercle*, **66,** 187.

KOCHAN I. (1973) The role of iron in bacterial infections, with special consideration of the host-tubercle bacillus interaction. *Curr. Top. Microbiol. Immunol.* **60,** 1.

KOEFFLER H.P., REICHEL H., BISHOP J.E. & NORMAN A.W. (1985) γ-Interferon stimulates production of 1,25-dihydroxyvitamin D3 by normal human macrophages. *Biochem. biophys. Res. Com.* **127,** 596.

MANGELSDORF D.J., KOEFFLER H.P., DONALDSON C.A., PIKE J.W. & HAUSSLER M.R. (1984) 1,25 dihydroxy vitamin D3-induced differentiation in a human promyelocytic leukaemia cell line (HL60): receptor-mediated maturation to macrophage-like cells. *J. Cell. Biol.* **98,** 391.

MIYAURA C., ABE E., KURIBAYASHI T., TANAKA H., KONNO K., NISHII Y. & SUDA T. (1981) 1,25 dihydroxy vitamin D3 induces differentiation of human myeloid leukaemia cells. *Biochem. Biophys. Res. Com.* **102,** 937.

NARANG N.K., GUPTA R.C. & JAIN M.K. (1984) Role of vitamin D in pulmonary tuberculosis. *J. Assoc. Physic. India*, **32,** 185.

ROOK G.A.W. (1985) The immunopathology of tuberculosis. In: *Proceedings of the International Symposium on Mycobacteria of Clinical Interest, Cordoba, Spain.* Elsevier, Amsterdam (in press).

ROOK G.A.W., CHAMPION B.R., STEELE J., VAREY A.M. & STANFORD J.L. (1985a) I-A restricted activation byT cell lines of anti-tuberculosis activity in murine macrophages. *Clin. exp. Immunol.* **59,** 414.

ROOK G.A.W., STEELE, J., UMAR S. & DOCKRELL H.M. (1985b) A simple method for the solubilisation of reduced NBT, and its use as a colorimetric assay for the activation of human macrophages by Ganna interferon. *J. immunol. Meth.* **82,** 161.